Use of Quantitative Light-induced Fluorescence to monitor tooth whitening

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ABSTRACT

The changing of tooth shade by whitening agents occurs gradually. Apart from being subjective and affected by the conditions of the surroundings, visual observation cannot detect a very slight change in tooth colour. An electronic method, which can communicate the colour change quantitatively, would be more reliable. Quantitative Light-induced Fluorescence (QLF) was developed to detect and assess dental caries based on the phenomenon of change of autofluorescence of a tooth by demineralisation. However, stains on the tooth surface exhibit the same phenomenon, and therefore QLF can be used to measure the percentage fluorescence change of stained enamel with respect to surrounding unstained enamel. The present study describes a technique of assessing the effect of a tooth-whitening agent using QLF. This was demonstrated in two experiments in which either wholly or partially stained teeth were whitened by intermittent immersion in sodium hypochlorite. Following each immersion, the integrated fluorescence change (ΔQ) due to the stain was quantified using QLF. In either situation, the value of ΔQ (%.mm^2) decreased linearly as the tooth regained its natural shade. It was concluded that gradual changing of the shade of discoloured teeth by a whitening agent could be quantified using QLF.

Keywords: sodium hypochlorite, tooth stain; tooth color, stain formation, salivary pellicle, stain removal, quantitative light-induced fluorescence; tooth whitening, tooth bleaching, optical methods

1. INTRODUCTION

The appearance of the teeth is very important for the majority of people, and any discoloration will affect their aesthetic qualities. Improvement of the appearance of discoloured teeth using whitening agents is one of the treatment modalities in dentistry, but the major problem associated with this treatment procedure is the difficulty in monitoring the change in colour, which occurs gradually and sometimes unnoticed. Standard shade guides, which require subjective visual grading, are used in most cases, but colour perception of the human eye is affected by various factors, which includes ambient lighting, surrounding colours and interpretation of individual assessors[1]. Visual observation, therefore, is not only subjective but cannot detect a very slight change in tooth colour. To overcome these difficulties and provide an accurate monitoring of stain removal by whitening agents, a computer-aided method that is not affected by the above factors, and which can accurately and quantitatively communicate colour change would be more reliable.

Spectrophotometry which determines color change by measuring the optical density of stained clear acrylic specimens has been used in *in vitro* studies[2, 3] to investigate the effect of whitening toothpastes, however this equipment cannot be used *in vivo*. Reflectance spectrometry such as the Minolta Chroma Meter (Minolta, Camera Co. Ltd, Japan) has been use in some studies[4, 5]. The use of this equipment by clinicians is limited by the fact that a series of calculations with standard equations is required before the final quantitative data can be obtained. Furthermore, the instrument measures the light reflected from the tooth surface being examined, so to obtain an accurate and reproducible data, color reading can only be taken on a flat surface of the tooth so that the aperture can be positioned as to prevent loss of reflected light and interference from external light sources. It was therefore necessary to develop a method that can be used in dental clinics to provide direct and instant quantitative evaluation of tooth color in addition to being consistent and accurate.

Quantitative Light-induced Fluorescence (QLF) is an optical technique used in caries diagnosis for detection and quantification of early caries lesions in enamel[6]. It uses the natural fluorescence of teeth to discriminate between caries and sound enamel based on the fact that the fluorescence radiance of a carious spot viewed with QLF is lower than that of

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surrounding sound enamel. Carious lesions appear dark when viewed with QLF, however stain on the tooth surface exhibit the same phenomenon and appear dark, similar to caries, and the darkness increases as the intensity of the stain increases. It was therefore envisaged that QLF might be capable of assessing stain removal using a whitening agent. The aim of the present study was to demonstrate the use of this equipment to quantify the gradual change in colour of stained teeth following the application of a whitening agent.

2. MATERIALS AND METHODS

2.1 Tooth preparation and staining

Freshly extracted teeth were collected, cleaned of all debris and soft tissue and examined. Twenty teeth free from caries, cracks or enamel malformations were selected and polished with pumice slurry (Associated Dental Products Ltd, Swindon, UK) to remove organic contaminants from the buccal surface. Ten teeth were then painted with two coats of an acid-resistant colorless nail varnish (Max Factor®, Procter and Gamble Int. UK), except for a window of exposed enamel (7 mm diameter) on the buccal surface. Acquired salivary pellicle was formed on all twenty teeth by gentle rotation (10 rpm) of the teeth for 2 h in human whole saliva using a rotary mixer (Sandrest, East Sussex, England). Following pellicle formation, the teeth were stained by 1 h immersion in 0.2% chlorhexidine gluconate mouthrinse followed by 4 h storage in a standard tea solution. The whole staining process was repeated twice before the teeth were finally stored overnight in tea. Boiling 6 gm of tea leaves in 500 ml of water for 2 minutes produced the tea solution which was then filtered through gauze to remove the leaves and then allowed to cool to room temperature. Both pellicle formation and staining were performed at room temperature (approximately 20°C). Following staining, the nail varnish on the painted ten teeth was removed with acetone (British Drug House (BDH), Poole, England) leaving only the window of exposed enamel with stain (Figure 1a). This therefore gave two experiments of ten teeth each. Experiment A consisted of ten teeth with a single spot stain (Figure 1a), while experiment B consisted of ten sporadically stained teeth (Figure 1b). All the teeth were subsequently mounted in a dental periapical radiograph film-holder to facilitate simultaneous immersion into a whitening agent.

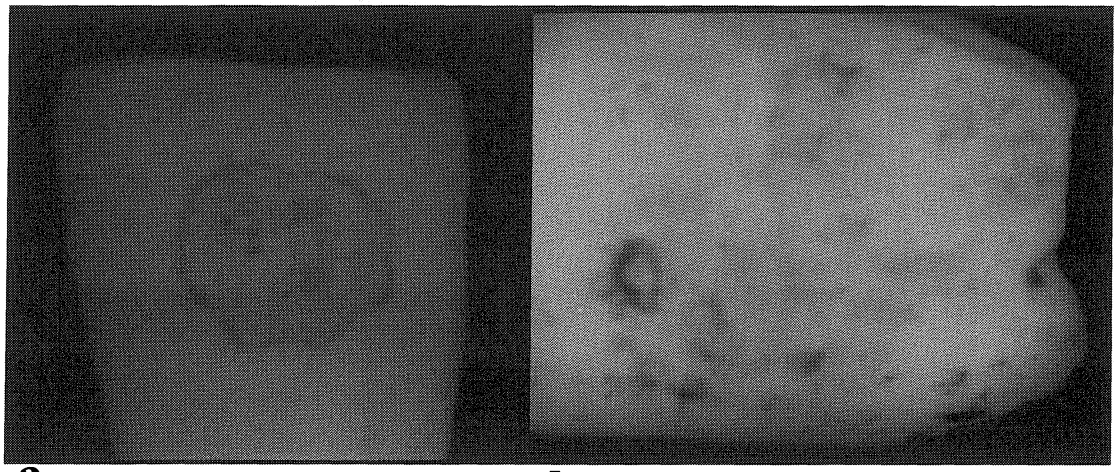


Figure 1: Fluorescent images of teeth with spot stain (a) and sporadic stain (b) captured with QLF.

2.2 Whitening and stain quantification

Prior to whitening, the fluorescent image of each tooth was captured using the QLF clinical system and stored on the computer (PC) for later analysis. The QLF system comprised of a special intra-oral camera device connected to a computer fitted with a framegrabber and to which the QLF software was installed (Figure 2). To visualise and capture the tooth image,

white light from a special arc lamp (based on Xenon technology) was filtered through a blue-transmitting bandpass filter (peak intensity of $\lambda = 370$ nm) to provide illumination of the tooth with a blue-violet light. A dental mirror provided uniform illumination of the tooth, and with the aid of a color CCD-sensor (Sony LS-1P), which had a yellow-transmitting filter ($\lambda \geq 520$ nm) positioned in front of it (to filter out all reflected and back-scattered light), the fluorescent image of the tooth was recorded and digitized by the framegrabber and was available for quantitative analysis with the QLF software[7]. This baseline recording was followed by gradual whitening of the teeth by intermittent immersion in 1:10 dilution of sodium hypochlorite (12% w/v available chlorine; BDH, Poole, England) for 60 sec on each occasion. Following each immersion, the teeth were air-dried and the images captured with the QLF system and recorded in the PC for later analysis. This procedure was repeated several times until one specimen in each experiment was observed by visual examination to have regained its natural color, with a total exposure number of six and four times for experiment A (Figure 1a) and experiment B (Figure 1b) respectively.

The parameters measured by QLF software are fluorescence radiance loss (ΔF in %), stained area (mm^2), and the stain intensity (ΔQ in $\%.mm^2$) which is the integrated fluorescence radiance change (Defined as the fluorescence radiance loss integrated over the stain area)[8]. The fluorescence loss in the stain was calculated according to the method described by de Josselin de Jong *et al.*[9], by reconstruction of the fluorescence radiance of sound enamel at the site of the stain from the fluorescence radiance of the surrounding sound enamel (assumed to be 100%). The decrease in fluorescence was determined by calculating the percentage difference between actual and reconstructed fluorescence surface. Any area with a fluorescence radiance drop of more than 5% is considered to be stained[6].

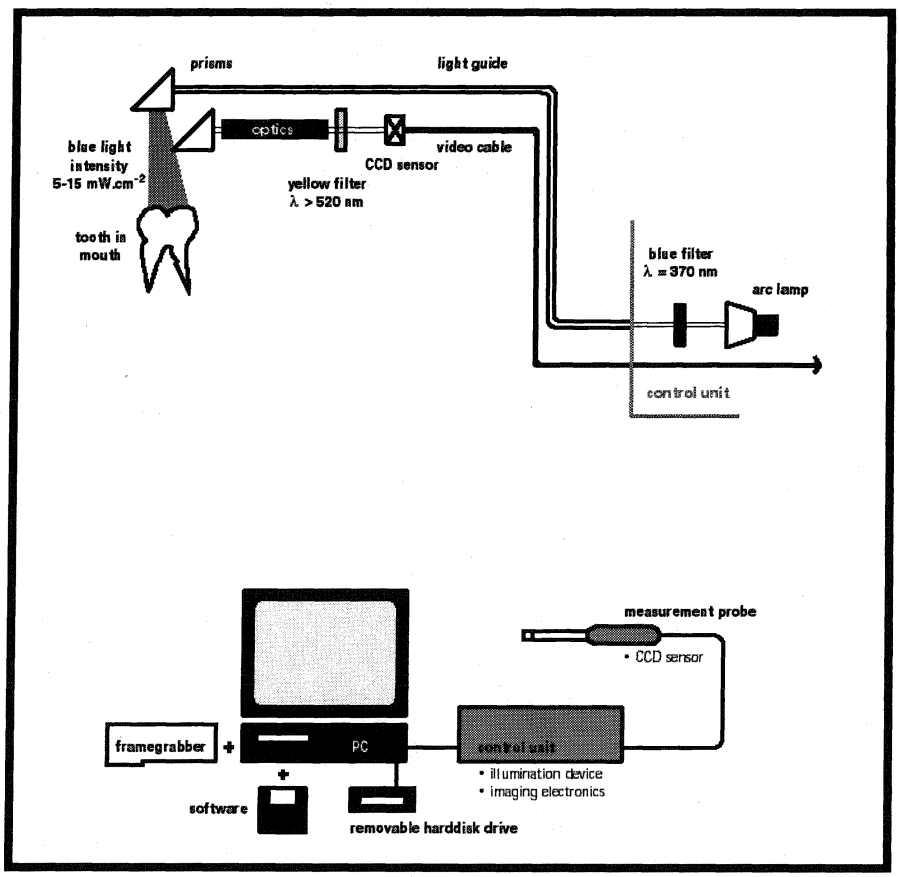


Figure 2: Schematic illustration of the QLF clinical system. The arc lamp and blue filter are housed within a portable illumination device (Control unit). The CCD sensor, yellow filter, prisms and a dental mirror are all contained within a hand-held intra-oral camera device (Measurement probe). The blue- and yellow-filter combination is optimized in such a way that the video image is completely free of reflections (Figure 4a,b). Courtesy of Inspektor Research Systems BV, Amsterdam, The Netherlands.

2.3 Statistical Analysis

For the monitoring of tooth whitening, only the ΔQ (%.mm^2) parameter which is a measure of the stain intensity was used for statistical analysis. The data obtained was analysed statistically by two-way analysis of variance (ANOVA) with significance level (α) prechosen at 0.05.

3. RESULTS

The mean variation in integrated fluorescence radiance change (ΔQ) with time as measured with the QLF clinical system is showed in Figure 3 a and b for experiment A and B respectively. In both experiments ΔQ decreased linearly as the stain intensity decreases with whitening time. Analysis of variance showed statistically significant differences ($p < 0.001$, $n = 10$) among the reading groups in both case.

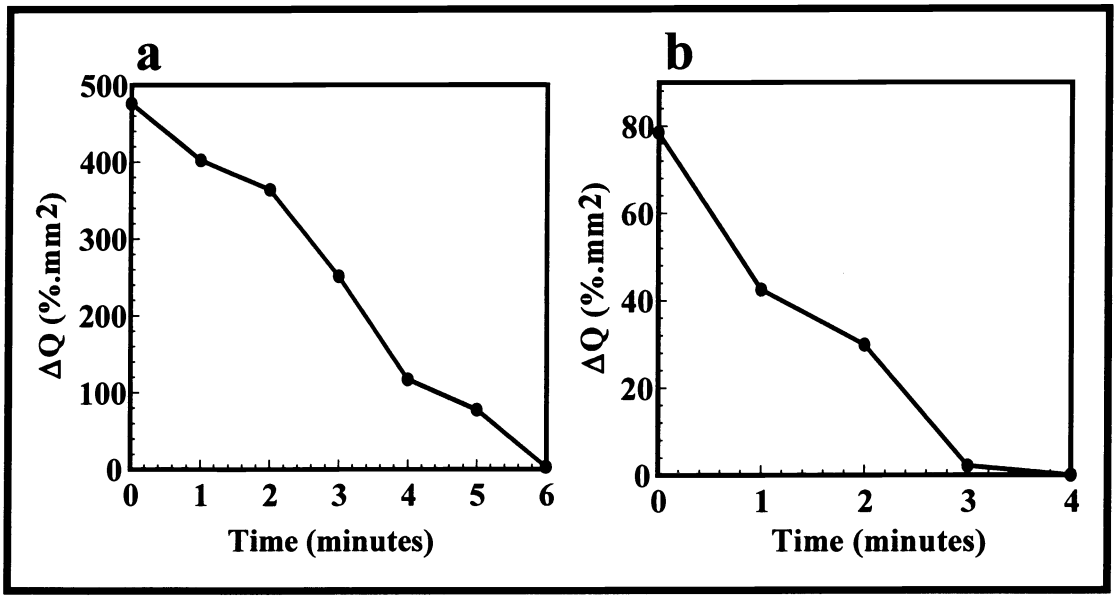
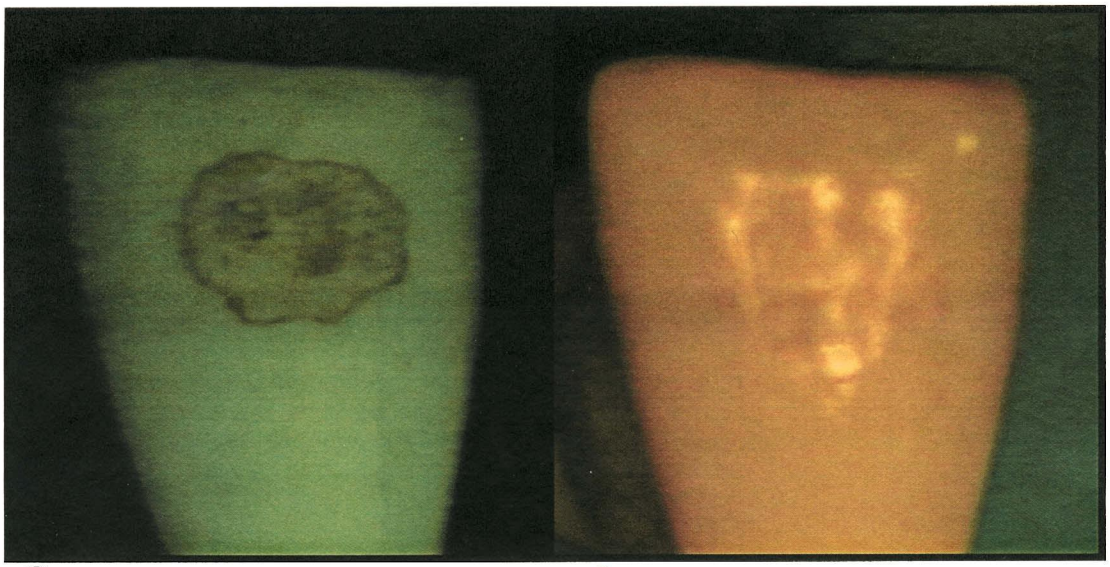


Figure 3: Graphical illustration of the change in stain intensity (ΔQ) with whitening time as measured by the QLF clinical system. **a** = experiment A with single-spot stained teeth (Figure 1a), **b** = experiment B with sporadically stained teeth (Figure 1b).

4. DISCUSSION

The use of QLF to monitor and quantify the whitening of discolored teeth, which was successfully demonstrated by the present study (Figure 3a, b), is an extension of the function of a technique which has been reported in several studies as a valid and reproducible method for the detection and quantification of early caries lesions in enamel[6, 10,11]. The sensitivity of this system in monitoring mineral changes in caries lesions with time, has been validated with previously used caries evaluation methods such as chemical analysis, transverse microradiography, quantitative laser fluorescence[10] as well as longitudinal microradiography[12]. QLF uses the same principle by which it depicts an early caries as a dark spot on the fluorescence image of the tooth to show a stain on tooth surface with similar appearance. As a result a stained spot on tooth surface viewed with QLF will appear dark, similar to a caries lesion (Figures 1a,b). QLF uses the natural fluorescence of the teeth, which is determined by the light absorption and scattering properties of teeth, to discriminate between caries and surrounding sound enamel[6]. Applying the same principle for stained enamel surface, it is assumed that stain limits the penetration of light, resulting in more scattering of photons entering stained enamel surface, with consequent limitation to the chance of a photon being absorbed and fluorescence remitted from the stained surface than from surrounding sound

surface. Hence we see a dark spot surrounded by highly luminescent sound enamel (Figures 1a,b) when a stained surface is viewed with QLF. The dark spot, whether caries or stain, is quantitatively analysed in exactly the same way by QLF software. Once the fluorescent image of the tooth has been captured and recorded by the PC, analysis of the stain can be initiated by a user-defined patch with borders placed on sound enamel surrounding the lesion, and the software automatically gives the values for the parameters of fluorescence radiance loss (ΔF), stained area (mm^2), and the stain intensity (ΔQ) with a simultaneous data storage[9]. Instantaneous provision of a quantitative data gives QLF an advantage for use in dental clinics over other existing methods of color measurements[4, 5]. Furthermore, the blue- and yellow-filter combination in QLF is optimized in such a way that the video image is completely free of reflections (Figure 4a,b), thereby making stain or caries detection easier and quicker. QLF detected 9.5 times more demineralised surfaces than visual clinical examination[13]. It can be seen from figure 4b that the reflections on the white light image of the tooth have obscured the stain, while the QLF image is completely free of reflections making the stain very evident (Figure 4a). It was surprisingly observed in the present study that QLF was able to analyse sporadically stained tooth (Figure1b and 3b), possibly making use of the intervening sound enamel surfaces. However, the system is currently being developed to be able to make use of a patch from a distant sound enamel surface for analysis of a homogeneously stained tooth.



Figures 4: Comparison of the image of a stained tooth viewed with QLF (a) and white light (b). Note the reflections on the white light image, which obscured the stain. The QLF image is completely free of reflections making stain detection easier than with white light. The yellow-transmitting filter ($\lambda \geq 520$ nm) positioned in front of CCD camera filters out all reflected and back-scattered light, and in combination with the blue bandpass filter, the video image is completely free of reflections.

Although the discoloration of teeth from extrinsic sources has been ascribed to a wide variety of substances[14], the stain formation technique used in the present study has been used for many years in dental research[2, 3,15]. The stain developed is almost entirely organic, representing discoloration that would develop initially *in vivo*. There has been a controversy over the mechanism of stain formation by dietary substances. The more conclusive evidence to date tends to favor the precipitation of chromogenic dietary compounds onto locally adsorbed antiseptic cations[16]. However, an ionic exchange type of mechanism has been reported to predominate, where ions on the pellicle surface are simply exchanged for those contained in foods or beverages[5], with chromophore retention occurring as a result of electrostatic attraction[17] and is easily removed by surface-active agents[18]. The whitening agent, sodium hypochlorite (NaOCL), used in the present study is an active bleaching agent, which acts by direct oxidation of the stain through its ability to release nascent oxygen[19].

5. CONCLUSIONS

The results of the present study demonstrated that the QLF clinical system is a suitable tool for quantitative monitoring of the gradual changing of the shade of discolored teeth by a whitening product, and will be useful in dental clinics for clinical monitoring of the effects of various tooth whitening products. It can be used in both laboratory (*in vitro*) and clinical studies (*in vivo*) for quantification of the stain removal efficacy of different tooth whitening agents.

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